Final Report for Period: 09/2006 - 08/2007 **Submitted on:** 11/29/2007
Principal Investigator: Dickson, Robert M. **Award ID:** 0323453
Organization: GA Tech Res Corp - GIT
Title:
Biophotonics: Genetically encodable single molecule fluorescence: peptide-silver nanodot biolabels

Project Participants

Senior Personnel

Name: Dickson, Robert
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Tzeng, Yih-Ling
Worked for more than 160 Hours: Yes
Contribution to Project:

Post-doc

Graduate Student

Name: Gonzalez, Jose
Worked for more than 160 Hours: Yes
Contribution to Project:
Performed optical measurements on dendrimer-encapsulated noble metal nanoclusters

Name: Capadona, Lynn
Worked for more than 160 Hours: Yes
Contribution to Project:
Chemical preparation of noble metal nanoclusters

Name: Zheng, Jie
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Koh, Wonsang
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Choi, Sungmoon
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Xu, Kewei
Worked for more than 160 Hours: Yes
Contribution to Project:

Undergraduate Student

Technician, Programmer

Name: Scally, Chris
Worked for more than 160 Hours: Yes
Contribution to Project:

Other Participant

Research Experience for Undergraduates

Organizational Partners

Emory University School of Medicine
Dr. Yih-Ling Tzeng is a co-PI on this proposal. Her lab is at the Emory University School of Medicine

Other Collaborators or Contacts

Professor Jeff Petty, Furman University (Greenville, SC), and I have exchanged students to perform Fluorescence correlation spectroscopy experiments on Ag and Au nanoclusters. Dr. Petty spent a 1-year sabbatical in my laboratory and we are continuing our strong collaboration with his lab at one of the South's best undergraduate institutions. We are currently co-authoring two papers with Dr. Petty's group at Furman. One of these has been published in J. Phys. Chem. C, 2007. I serve as an external advisor to the Furman University/South Carolina INBRE program and have been interacting with Dr. Petty continuously through this mechanism. We plan a joint submission to NSF in the Spring.

Activities and Findings

Research and Education Activities:
We have designed new methods of creating spectrally pure gold and silver nanoclusters in a variety of biocompatible scaffolds. We continue to study their optical properties on both bulk and single molecule levels. These materials are being created with an eye toward biolabeling, but important fundamental scaling laws connecting the atomic and nanoparticle levels have been discovered.
I have given thirteen invited presentations on this work at both universities and national conferences within the past year.
One undergraduate, Ms. Caiwei Zhang, has been instrumental to the success of this project and is a co-author on one publication resulting from this project.
Our interaction with Prof. Jeff Petty at Furman University has enabled us to jointly measure extinction coefficients of our nanoclusters and involve some of his undergraduates in these studies. In addition to those already published, we are currently co-authoring 2 papers with Dr. Petty and the excellent undergraduates in his laboratory.

Findings:
We have created spectrally pure gold nanoclusters ranging from 5 to 31 atoms inside dendrimer hosts. We have identified which nanocluster size gives which color emission and they perfectly follow a jellium model scaling law with absorption and emission energies scaling linearly with the inverse of the nanocluster radius. This is a new scaling and makes these materials true 'multi-electron artificial atoms'. This is one of the major projects that an undergraduate, Ms. Caiwei Zhang, has contributed significantly to. We have determined that these have a factor of 10 stronger absorption than the best organic dyes and are indeed true multielectron artificial atoms (and complementary to much larger semiconductor quantum dots)
We have also created Ag nanoclusters in DNA and noticed a strong sequence dependence in its interaction with the individual bases. So far, we have created red and green fluorescent silver nanoclusters with very narrow cluster size distributions. We are currently intensively investigating the nanocluster photophysical properties.
Very recently, we have even observed and characterized single molecule Raman spectra from the scaffold encapsulating the individual Ag nanocluster. In contrast to all other single molecule Raman studies, no large nanoparticle is necessary to observe such signals. This observation may enable very high sensitivity Raman-based biolabeling. This last work was published in Phys. Rev. Lett, 94, 058301 (2005), and cites NSF funding.
We have identified 18-aa peptides capable of encapsulating small Ag nanoclusters and observed these to be transported across cell membranes. We have been able to observe Ag nanocluster fluorescence inside live and fixed cells. These peptides are sub-optimal and need to be optimized. These are being optimized through bead-based split-pool libraries coupled with flow cytometry, but using the newly identified

18-mer sequence as a starting point. This peptide sequence, itself, was based on a silver binding protein in the nucleolus. While these peptides do not stabilize the ultrabright Ag emission, we have had success creating such ultrabright emitters with tyrosine-mediated photoreduction and small peptides for encapsulation and separation to prevent large nanoparticle formation.

Further, encapsulation with oligonucleotides has been most successful. Ag nanoclusters in ss-DNA exhibit bright, non-blinking, highly photostable emission. We have created a ~710-nm emitter (published in PNAS, July, 2007), as well as spectrally pure emitters at 650nm, 630nm, 570nm, 525nm, and 485nm. We are studying the photophysics of all of these emitters in bulk and single molecule levels. The latter studies have been done in buffer when attached to proteins.

Training and Development:

Ms. Caiwei Zhang (Georgia Tech undergraduate) has learned the joys and difficulties of research. She has learned how to effectively present her research and how to create and characterize new nanomaterials.

Graduate students are going to either Materials research society or gordon conferences to present their work. One student, Mr. Jie Zheng was a Silver Medallist in the Materials Research Society Graduate Student Contest. They collectively work on these projects and teach each other synthetic and optical skills. The students are beginning to unravel the nature of the atom to nanoparticle transition and how new, unimagined properties can be conferred upon normally ordinary materials when confined to the nanoscale.

The students are also deciphering the effect of the chemically stabilizing scaffold (dendrimer, peptide, DNA) on the noble metal nanoclusters.

Ms. Lynn Peyser and Mr. Jie Zheng both graduated with their PhD's during this most recent funding period. Dr. Peyser is now a research scientist at NASA in Cleveland, OH and Dr. Zheng is a postdoctoral researcher at Harvard. Several new students have joined the group and are investigating a variety of peptides and oligonucleotides as optimized scaffolds for metal nanocluster labels.

Students in Dr. Tzeng's lab at Emory have begun constructing and screening bacterial libraries for peptide binding to silver nanoclusters. They are also systematically investigating array-based methods for greatly improving chemical yields of fluorescent noble metal nanoclusters. Two of these students are currently attending medical school.

Outreach Activities:

Journal Publications

Vosch, T; Antoku, Y; Hsiang, JC; Richards, CI; Gonzalez, JI; Dickson, RM, "Strongly emissive individual DNA-encapsulated Ag nanoclusters as single-molecule fluorophores", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, p. 12616, vol. 104, (2007). Published, 10.1073/pnas.061067710

Zheng, J; Nicovich, PR; Dickson, RM, "Highly fluorescent noble-metal quantum dots", ANNUAL REVIEW OF PHYSICAL CHEMISTRY, p. 409, vol. 58, (2007). Published, 10.1146/annurev.physchem.58.032806.10454

Lee, TH; Gonzalez, JI; Zheng, J; Dickson, RM, "Single-molecule optoelectronics", ACCOUNTS OF CHEMICAL RESEARCH, p. 534, vol. 38, (2005). Published, 10.1021/ar040146

Peyser-Capadona, L; Zheng, J; Gonzalez, JI; Lee, TH; Patel, SA; Dickson, RM, "Nanoparticle-free single molecule anti-stokes Raman spectroscopy", PHYSICAL REVIEW LETTERS, p. , vol. 94, (2005). Published, 10.1003/PhysRevLett.94.05830

Zheng, J; Zhang, CW; Dickson, RM, "Highly fluorescent, water-soluble, size-tunable gold quantum dots", PHYSICAL REVIEW LETTERS, p. , vol. 93, (2004). Published, 10.1103/PhysRevLett.93.07740

Petty, JT; Zheng, J; Hud, NV; Dickson, RM, "DNA-templated Ag nanocluster formation", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, p. 5207, vol. 126, (2004). Published, 10.1021/ja031931

Zheng, J; Petty, JT; Dickson, RM, "High quantum yield blue emission from water-soluble Au-8 nanodots", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, p. 7780, vol. 125, (2003). Published, 10.1021/ja035473

Zheng, J; Dickson, RM, "Individual water-soluble dendrimer-encapsulated silver nanodot fluorescence", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, p. 13982, vol. 124, (2002). Published, 10.1021/ja028282

Ritchie, CM; Johnsen, KR; Kiser, JR; Antoku, Y; Dickson, RM; Petty, JT, "Ag nanocluster formation using a cytosine oligonucleotide template", JOURNAL OF PHYSICAL CHEMISTRY C, p. 175, vol. 111, (2007). Published, 10.1021/jp064848

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Contributions

Contributions within Discipline:

We have discovered new size scaling relationships of transition energies for noble metal nanoclusters. These are well-described by the spherical jellium model and smoothly link the atomic and nanoparicle behaviors while giving strong size-dependent fluorescence at the several-atom scale. This indicates that metal nanoclusters even as small as 5 atoms exhibit metallic behavior.

We have also developed the unique ability to observe single molecule antistokes Raman spectroscopy of biocompatible scaffolds, without a field-enhancing nanoparticle. The small silver nanocluster is sufficient to enhance such signals. This has completely re-defined the field of single molecule Raman, with Prof. George Schatz (Northwestern) providing calculations that support our experimental observations that clusters provide the same enhancement as that seen with nanoparticles.

Both of these have outstanding photophysical properties and are likely to make excellent biolabels. We have created concentrated spectrally pure solutions of Ag nanoclusters encapsulated in ss-DNA. Tuning the DNA sequence affords control over nanocluster size and emission color while simultaneously conferring excellent chemical stability in buffer. We have attached these to proteins and shown they are excellent single molecule fluorescent labels, giving ~40x greater photostability and higher emission rates than the best available organic dyes.

Contributions to Other Disciplines:

The scaling relationships we've uncovered have important implications for cluster physics, biolabeling, and nanoscience. There is a great effort in understanding the properties of nanoscale (and smaller) metals, and in understanding how these properties can be predicted at small sizes where behavior is very different from the bulk.

We have also uncovered interactions giving rise to the large enhancemnents in single molecule Raman spectroscopy. These Raman-enhancing few-atom nanoclusters may be useful as novel biolabels, and may even enable sensing on the single molecule level.

These studies have important implications in nanoscience in terms of how properties change at the nanoscale, and also in biolabeling. In fact, these nanoclusters have recently been licensed by a biotech company with hopes of commercialization for biolabeling applications.

Contributions to Human Resource Development:

Contributions to Resources for Research and Education:

Once optimized creation methods are identified and absoprtion cross sections are determined for all nanoclusters (and therefore concentrations are known), we will incorporate nanoscale Au fluorescence experiments in the physical chemistry lab. As Georgia Tech has a very large minority student population, this will hopefully help encourage underrepresented minorities into science.

The interaction with Dr. Petty at Furman University exposes undergraduates to aspects of our research for biolabel development.

Contributions Beyond Science and Engineering:

Patents have been applied for based on the Ag and Au nanocluster fluorescence. A large biotech company has licensed our nanomaterials for bioassay development and biolabeling. It is through this mechanism that our nanomaterials should become available to all for general use.

Categories for which nothing is reported:

Activities and Findings: Any Outreach Activities

Any Book

Any Web/Internet Site

Any Product

Contributions: To Any Human Resource Development